

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 14-R-0009
CUSTOMER NUMBER: 105

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Boston University Medical School
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Boston, MA 02118

Telephone: (617) -638-4089

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reaso such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	0	0	0	0
5. Cats	0	11	25	0	39
6. Guinea Pigs	0	0	10	0	10
7. Hamsters	0	0	4	0	4
8. Rabbits	0	59	55	0	114
9. Non-human Primates	0	0	61	6	67
10. Sheep	0	0	0	0	0
11. Pigs	0	0	18	0	0
12. Other Farm Animals	0	0	0	0	0
13. Other Animals					
Chinchillas	0	0	142	0	142
Fennets	0	0	3	0	3

ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, testing, surgery, or experimentation were followed by this research facility.
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

(b)(6), (b)(7)c

SIGNED

4/05

RAG

Column E Explanation

This is the form that is submitted as an attachment to the Annual Report:

Column E Explanation Form- This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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- 1. Registration Number:** 14-R-0009
 - 2. Number of animals used under Column E conditions in this study.** six
 - 3. Species (common name) of animals used in this study.** Rhesus monkeys
 - 4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

The proposed housing conditions in Protocol # 14560 were determined by the Attending Veterinarian to require the animals enrolled in this project be classified as USDA Pain Category E. The IACUC was informed that it would be necessary to approve three exemptions from accepted standards of care for nonhuman primates, as described in the Guide for the Care and Use of Laboratory Animals (ILAR/NRC 1996), as scientifically justified if the protocol were to be approved. The three points of exemption related to: illumination, socialization and environmental enrichment. These conditions were considered to be distressful and could not be relieved by the use of appropriate anesthetic, analgesic, or tranquilizing drugs. The IACUC approved these exemptions because it determined the conditions to be scientifically justified and the use of drugs would adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

Exemptions:

- **Lighting.** This project involves housing animals in constant dim light (20 lux at cage top level) during constant condition parts of the study for two periods up to four months. During the remainder of the experimental period the animals will be in environmentally controlled housing on a 12:12 h light:dark cycle.
- **Socialization:** Throughout the entire protocol, the animals will be housed in specially designed individual sleep/circadian chambers that contain an over-sized steel primate cage. The chambers provide controlled levels and timing of environmental illumination, low noise

- level and limited intra- and inter-species interactions. By limiting inter-specific and intra-specific interactions, as well as exposure to facility noise to randomly scheduled times ensures that each animal's behavior reflects endogenous processes rather than responsiveness to the events in their environment.
- Environmental enrichment: Isolation of the animals from each other is a critical part of the experimental design when studying circadian rhythms. Sources of enrichment that partially compensate for lack of social interactions that are used in the Protocol include toys and mirrors in each cage and access to "in-house" computerized performance.

Nonhuman primates were selected for this protocol because:

- Technical and ethical issues preclude using human subject in the many types of studies that could help to elucidate the role of the suprachiasmatic nucleus of the hypothalamus (SCN) and the pineal gland in physiological aging and the mechanisms of melatonin action in humans. Thus it is necessary to conduct such studies using appropriate animal models. Animal models allow experiments to be conducted in the strictly controlled environmental conditions that are critical for any circadian rhythm study including maintenance of uninterrupted recording of circadian rhythms of locomotion, core body temperature, polysomnographically assessed sleep, cognitive performance and food intake under entrained (i.e., light-dark cycle) or constant conditions. Some aspects of cognitive performance, sleep and entrainment effects of melatonin treatment can be studied in humans kept in constant routine or forced desynchrony conditions, but obviously the assessment of the surgical procedures cannot be accomplished in human studies. Similarly, rodent models are inadequate as the role of the SCN in regulating sleep seems to be fundamentally different in rodents vs. primates, and voluntary performance of cognitive testing around the clock is impossible.
- There are several important similarities between humans and diurnal non-human primates favoring the use of these species to model alterations in sleep and the circadian system that occur in aging humans, and to study the mechanisms of melatonin sleep-promoting effects. 1. Phylogenetic proximity of these species; 2. Similar temporal patterns of activation of the major circadian pacemaker, SCN, relative to the rest-activity cycle in both species, i.e., high activity of the SCN neurons during the day correlates with these species daytime activity (in contrast to nocturnal animals whose SCN is active during their daytime rest period); 3. The nocturnal increase in melatonin production in both humans and in diurnal non-human primates occurs during their habitual sleep period (while in nocturnal species high plasma melatonin coincides with peak motor activity); 4. Both humans and diurnally active *Macaca* species display a consolidated nocturnal sleep episode, with a similar sleep architecture (Balsamo et al, 1977, 1988; Yamamoto et al., 1987; Lagarde & Milhaud, 1990), in contrast to the majority of nocturnal or diurnal species who tend to have a polyphasic sleep pattern; 5. The range of circulating melatonin concentrations and the changes that occur in melatonin

- levels during maturation are similar in the Macaca and humans (Reppert et al., 1981; Wilson & Gordon et al., 1989); 6. Non-human primates are sensitive to the sleep-promoting effects of physiological melatonin concentrations (Zhdanova et al., 1998a, 2000), a phenomenon that was previously observed only in humans (Dollins et al., 1994; Zhdanova et al., 1996) but not in nocturnal rodents (Mendelson, et al., 1980; Tobler, et al., 1994; Sugden, 1995); 7. A vast amount of data on the age-related changes in cognitive functions, cardiovascular system or brain morphology in humans show similarities with alterations observed in aging non-human primates (Zatz et al., 1982; Tang et al., 1997; Emborg et al., 1998; Peters et al., 1996, 1998); 8. Another important factor that increases the value of non-human primates for sleep and circadian rhythm studies is that in diurnal non-human primates (as in humans but in contrast to nocturnal animals) the SCN assures sleep-wake cycle consolidation by actively promoting wakefulness: the SCN-lesioned (SCNx) monkeys show a significant reduction in subjective day wake consolidation, as evidenced by substantially shorter wake bout lengths in SCNx monkeys and increase in total sleep time, as compared to intact animals (Edgar et al., 1993). Such effect was not observed in studies with nocturnal SCNx species. These inconsistencies might be related to the basic differences in the signaling pathways downstream of the SCN in nocturnal and diurnal animals. Use of diurnal rhesus monkeys should help to answer important basic questions of circadian physiology and develop therapeutic strategies to address age-related alterations in the circadian system.
- Furthermore, a systematic study comparing thirteen non-human primate species (Balsamo 1977) determined that the rhesus monkey was the best single model because of its well-defined sleep organization, and the volume of anatomical, behavioral and physiological data known about this species. Importantly, the rhesus monkey has brain structure and behavioral capacities sufficiently similar to humans to allow immediate extrapolation of the research findings to the human conditions, including changes in cognitive functions.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

The results of the circadian studies are purely statistical, since multiple factors are involved in daily behaviors, and no conclusion can be made regarding a circadian rhythm of cognition or sleep based on few data points collected over several days. That is why all the circadian studies are inherently long and are conducted continuously and in extremely well controlled conditions in order to reduce their duration, while collecting sound scientific data.

Nonstandard lighting and socialization can not be relieved nor can standard methods of environmental enrichment be provided because of the requirement for constant dim lighting and isolation in circadian chambers which allow each animal to express and maintain individualized circadian pattern of activity, sleep and feeding, without interference from the facility noise or

other monkeys. On each given day, animals eat or sleep, or do cognitive tests at different hours. Thus, experimental results can be based on the statistical analysis of a large data set that may reveal common patterns between many consecutive days of uninterrupted and undisturbed recordings in a statistically significant number of animals. These animals will be studied in both standard light-dark cycle and constant dim light conditions while being socially isolated and deprived of traditional environmental enrichment.
